A clinical study on the relationship between dentures and oral Candida species

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Abstract: Purpose: The purpose of this study is to clarify the relationship between the wearing of dentures and oral Candida species.

Subjects: The subjects comprised 417 patients who visited the Kawasaki Dental and Oral Surgery Clinic during the 18 months from February 2007 to August 2008, exhibited no findings of oral candidiasis, and provided informed consent for the purpose of this study.

Methods: All of the subjects were surveyed regarding their age, sex, and status of denture use, and as samples, we collected swabs from the dorsum of the tongue from all of the subjects, swabs from the mucosa below the denture base from 76 denture wearers (143 bases), and the ultrasonic cleaning fluid of the dentures of 25 patients (42 bases) who had worn dentures. The collected samples were seeded and cultured on CHROMAGAR CANDIDA® medium to detect and identify C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, and C. krusei.

Results: The detection rate of Candida species was significantly high among females, elderly patients, and denture wearers in the swabs from the dorsum of the tongue and among patients wearing maxillary dentures compared to those wearing mandibular dentures in the swabs from the mucosa below the denture base. When measured by the site of sample collection, the detection rate of Candida species from the ultrasonic cleaning fluid of the dentures exhibited significantly higher values compared to the swabs from the dorsum of the tongue and the mucosa below the denture base, and regarding the bacterial species, it was found that non-albicans Candida species such as C. glabrata were dominant.

Conclusion: The results of this study indicated a relationship between the wearing of dentures and oral Candida species and indicated that non-albicans Candida species were dominant in the samples of denture cleaning fluid.

Key words: oral candida species, denture, oral candidiasis, denture stomatitis, non-albicans Candida species

Introduction

With the rapid aging of our society, the inappropriate use of antibiotics, and the heavy usage of immunosuppressive drugs and the like, oral mucosal diseases are becoming diverse and intractable cases with indefinite complaints such as oral dryness, tongue pain, halitosis, and taste disorders are frequently encountered1, 2). It has been believed that oral candidiasis is often developed as pseudomembranous candidiasis that is due to endogenous infections caused by resident oral Candida species and forms prominent white patches.
in elderly patients and patients with decreased immune strength\textsuperscript{3}. However, in recent years, there have been an increasing number of reports of erythematous candidiasis mainly involving erythema. Erythematous candidiasis, also known as atrophic candidiasis or denture stomatitis, has been understood as a type of oral candidiasis that develops only among denture wearers, but recently, it has also developed in cases of non-denture wearers as well and is recognized as a type of oral candidiasis similar to hypertrophic candidiasis and the like. Furthermore, intractable cases of desquamative cheilitis and angular cheilitis for which steroid drugs are ineffective are handled as Candida-related diseases, and even if typical symptoms of oral candidiasis, such as the formation of white patches, are not expressed, it has been indicated that resident oral Candida species may cause discomfort in the oral cavity\textsuperscript{2, 4, 5}).

We observed that the detection rate of Candida species in the oral cavities of denture wearers was high, and there was a significant positive correlation between the detection rate of Candida species and the number of missing teeth\textsuperscript{6}). Therefore, in this study, we expanded the number of cases to include non-denture wearers among the subjects in order to clarify the relationship between the wearing of dentures and the detection rate of Candida species while also investigating the state of distribution of oral Candida species and their state of habitation inside the denture base.

Subjects and Methods

1. Subjects
The subjects comprised 417 patients who visited the Kawasaki Dental and Oral Surgery Clinic during the 18 months from February 2007 to August 2008, exhibited no obvious findings of oral candidiasis.

2. Informed consent and ethical guideline
The subjects were patients from whom informed consent was obtained upon providing verbal and written explanations of the details of this investigation directly to the subject. This study is compliant with Kagoshima University Hospital Clinical Ethical Guideline and permitted to do by Kagoshima University Hospital Clinical Ethical Review Board (No.34, No.65).

3. Methods
After removing the dentures of denture wearers, rinsing out the mouth with tap water for 10 seconds, and cleaning the mucosal surface of the denture base under running water for 60 seconds, swabs were obtained from the dorsum of the tongue from all of the subjects and from the mucosa below the denture base from the denture wearers. Furthermore, for subjects who wore dentures, after cleaning the mucosal surface of the denture base with a denture brush for 60 seconds, the dentures were immersed in a sterile normal saline solution in a sterile beaker to undergo 5 minutes of ultrasonic cleaning and the cleaning fluid (hereinafter “cleaning fluid”) was collected. Sterile disposable plastic loops were used for sample collection, and the samples of each subject were seeded on a CHROMAGAR CANDIDA\textsuperscript{®} medium produced by B&D Co. Japan, for 48 hours at 36°C. Moreover, Candida albicans, C. glabrata, C. tropicalis, C. parapsilosis, and C. Kursei were identified based on the color tone of the colonies\textsuperscript{5}) and the detection rates of the Candida species were examined (Fig. 1). Furthermore, we investigated the relationships between these results and the sex, age, and status of denture use of the subjects.

4. Statistical analysis
For two-group comparisons of parametric data, a normal distribution test and a t-test were performed, and for tests of independence of non-parametric data, a chi square test was performed, and tests for statistically significant differences were examined (p<0.05).

Results

1. Case details
The subjects comprised 417 patients (156 males, 261 females), and there were more females than males. Swabs were obtained from the dorsum of the tongue from all of the subjects. There were 258 denture wearers (95 males, 163 females) and 159 non-denture wearers (61 males, 98 females), but there was no significant bias in the relationship between the status of denture use and sex. Swabs from the mucosa below the denture base were collected from 76 of the 258 denture wearers (143 bases), including 26 males (50 bases) and 50 females (93 bases). The ultrasonic cleaning fluid of the dentures was collected from 25 subjects...
(42 bases) who received dentures from among the 76 subjects from whom swabs could be collected from the mucosa below the denture base, including 9 males (14 bases) and 16 females (28 bases) (Table 1).

2. Sample culture results

(i) Comparisons of culture results of swabs from the dorsum of the tongue

The detection results of Candida species from the swabs from the dorsum of the tongue exhibited no significant differences by age, but there were significantly more female subjects testing positive for Candida species (Table 2-1). Regarding the status of denture use, the denture wearers were significantly older than the non-denture wearers and there were significantly more denture wearers testing positive for Candida species (Table 2-2). In a comparison of the mean ages by culture results, the subjects testing positive for Candida species were 66.8 years old on average, which

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There was no significant bias in the relationship between the status of denture use and sex.

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ultrasonic cleaning fluid for dentures

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was significantly higher compared to the subjects testing negative for Candida species (Table 2-3).

(2) Comparison of culture results of swabs from the mucosa below the denture base

In a comparison of the detection results of Candida species in the cultured swabs from the mucosa below the denture base, there were no significant differences by sex and the age. There was no significant difference by age in the status of denture use, but in the culture results, there were significantly more maxillary denture wearers testing positive for Candida species. In a comparison of the mean ages by culture result, the subjects testing positive for Candida species were 74.4 years old on average, which was higher compared to the subjects testing negative for Candida species (Table 3).

(3) Comparison of culture results of ultrasonic cleaning fluid for dentures

In a comparison of the detection results of Candida species in the cultured ultrasonic cleaning fluid of the dentures, the females were significantly older than the males but there was no significant difference in the culture results. There were no significant differences
The detection rate of Candida species by collected sample was highest in the ultrasonic cleaning fluid of the dentures at 73.8%, following by the swabs from the dorsum of the tongue at 58.5% and the swabs from the mucosa below the denture base at 47.6%. In the comparison of detection rates of Candida species between mucosa below the denture base and ultrasonic cleaning fluid for denture, statistically significant differences were observed between each group (Table 5).

In the ultrasonic cleaning fluid for dentures, C. albicans exhibited a high value but C. glabrata was the dominant species.

In the comparison of detection rates of Candida species between mucosa below the denture base and ultrasonic cleaning fluid for denture, statistically significant differences were observed between each group (Table 5).

4. Collected samples and detected Candida species

In the detection rates of Candida species in each of the collected samples related to the 25 subjects (42 bases) who wore dentures, in the swabs from the dorsum of the tongue, C. albicans was the dominant species, constituting the majority of the detected species at 52.4%, and in the waste fluid from the ultrasonic denture washing, C. albicans exhibited a high value of 36.1% but C. glabrata was the dominant species at 38.9% (Table 6).
Discussion

Regarding the relationship between dentures and Candida species in the oral cavity, there are reports that Candida species were detected at significantly higher rates on the tongue surface and in the saliva of denture wearers compared to non-denture wearers\(^7, 8\). We have also reported that a high detection rate of Candida species of 75.9% was observed in denture wearers with no findings of oral candidiasis\(^6\), and in this study as well, there were significantly more denture wearers testing positive for Candida species in the comparison of the culture results of the swabs from the dorsum of the tongue. Furthermore, there was a significant difference in the mean ages of the two groups. According to previous literature, there is no relationship between age and the Candida carriage rate, and there are reports that denture stomatitis is related only to the status of use of dentures\(^9\). In this study, in the results of the comparison of the culture results of the swabs from the mucosa below the denture base, the mean age of the subjects testing positive for Candida species was approximately 3 years older than that of the subjects testing negative for Candida species and a statistically significant difference was observed, thus indicating that there is a need to consider the effects of age.

Moreover, in the comparison of the culture results of the swabs from the dorsum of the tongue, a gender difference was observed, as there were a statistically significantly higher number of female subjects testing positive for Candida species, but we were unable to find any reports regarding gender differences in the Candida carriage rates in the oral cavities of healthy subjects. Factors affecting the carriage rate of oral Candida species include the status of denture use, age, amount of salivation\(^9\). Moreover, in this study, there were no significant biases between the males and females in terms of status of denture use or age (Tables 1, 2). Regarding the amount of salivation, Itoh et al.\(^10\) have reported that at least 70% of outpatients with dry mouth are females who complain of oral dryness at a high rate, indicating a relationship with the Candida carriage rate in females.

Regarding the habitation sites of oral Candida species, in this study, we examined the dorsum of the tongue, the mucosa below the denture base, and the ultrasonic cleaning fluid of the dentures and observed statistically significant differences between each of the groups, wherein the highest amount of Candida species was detected in the ultrasonic cleaning fluid of the dentures. When an abundance of moisture and oxygen and sufficient nutrients such as sugar and iron are provided, Candida species form a biofilm at sites that are not highly susceptible to the defense mechanisms of the oral cavity and become resident bacteria, and the site becomes a source of infection as the bacteria float inside the oral cavity through saliva and serum components to become aggregated with resident bacteria groups on organs inside the oral cavity, such as the tongue and the buccal mucosa, adhering and becoming settled to cause so-called endogenous infections and sometimes spreading to the gastrointestinal tract and the upper respiratory tract in patients susceptible to infections\(^3, 11\). Acrylic resin, which is used for various purposes as material for dentures, is relatively porous and has a high rate of water absorption, allowing a sufficient amount of moisture and nutritional components into the denture base\(^12\), indicating that the denture base is a site where oral Candida species become settled. Hamada\(^8\) have defined denture plaque as "biofilm that is formed on the surface of the denture and contains 10\(^{11}\)-10\(^{12}\) microorganisms per 1 g of wet weight", and it is considered the main cause of denture stomatitis. Regarding the bacterial composition, it has been reported that the denture plaque of denture stomatitis patients contained high amount of Candida species (0.25%), which is approximately 10 times higher than in control cases (0.02%)\(^9\). However, in our previous report\(^6\), no significant differences were observed in comparisons of Candida culture examinations from the mucosal floor of the denture, the mucosa below the denture base, and the dorsum of the tongue, and in denture wearers who had not developed denture stomatitis, there was no accumulation of Candida species on the surface of the denture base to form a plaque harbor. However, in this study, in the ultrasonic cleaning fluid of the dentures obtained after mechanically removing the denture plaque through brushing, Candida species were detected at a high rate of 73.8%.
Therefore, rather than biofilm on the surface layer of the denture, this reveals the presence of Candida species inside the denture material from the superficial layers to the deeper layers of the resin. According to the previous literature, there are similar reports that in an electron-microscope observation of denture plaque formed in vivo, bacterial threads of C. albicans infiltrated into the resin, albeit within a shallow range, and in addition, it is said that when resin fractions are immersed in vitro in C. albicans suspensions, adhesion to the surface occurs in 30 minutes and the C. albicans infiltrates into the resin within 4 hours\(^9\). These Candida species easily infiltrate into the resin, and there is concern that this may induce material deterioration while the resin also becomes a source of infection as a plaque harbor. As countermeasure, adjustments have been made, including improvements of denture materials using heat-curing resins such as Lucitone199\(^{13}\), surface finishing using rotational cutting\(^{14}\), and hydrophilic coating of the material surface using colloidal silica and the like\(^{15}\). The infiltration of oral Candida species into the material of the denture base is believed to be related to the ability to morphologically grow based on dimorphism and thigmotropism, wherein hypha are extended to the floor surface and the floor interior to obtain nutrition as typified by C. albicans\(^{16}\). In the medical field, central venous catheters are the most common infective focus of the bloodstream for exogenous infections of Candida species, wherein many of the originating bacterial species are non-albicans Candida species such as C. parapsilosis\(^{13}\) that are lowly susceptible to azole antifungal drugs such as fluconazole and cause disseminated deep candidiasis. The detection rate of C. glabrata is becoming particularly high among elderly patients, and in cases of neutropenia, caution is paid for non-albicans Candida species\(^3, 17\). In this study as well as in central venous catheters, non-albicans Candida species were detected at a high rate in the ultrasonic cleaning fluid of the dentures, and it was clarified that C. glabrata specifically is the dominant species.

Clinically, even when sufficient guidance is provided for denture cleaning and oral care to denture wearers, it is found that sufficient self-management is not being performed, and furthermore, patients requiring nursing care do not always implement sufficient hygienic management\(^{16}\). As clarified in this study, denture wearers had a high rate of Candida species in the oral cavity. In fact, even when there is not denture stomatitis, white patches, pseudomembrane formation, or the like, in many cases expressing clinical symptoms such as oral dryness, taste disorders, and tongue pain, Candida species are detected from inside the oral cavity, and cases indicative of inapparent infections of Candida species are encountered frequently. There is currently much discussion on the increase due to the rapid aging of society in patients susceptible to infections who have systemic complications as well as the expression of resistant bacteria due to the heavy usage of antibiotics and antimycotic drugs, and it is believed that in addition to hygienic management inside the oral cavity, the hygienic management of dentures, which are removable prosthetic appliances that may become sources of exogenous infection, is one of the tasks that we dentists have been assigned.

Conclusion

(1) In the cultures of the swabs from the dorsum of the tongue of all of the subjects, the detection rate of Candida species was statistically significantly higher among females, elderly patients, and denture wearers.

(2) In the comparison of detection rates of Candida species between mucosa below the denture base and ultrasonic cleaning fluid for denture, the detection rates of Candida species of ultrasonic cleaning fluid for denture was statistically significant higher.

(3) In the culture examinations of the ultrasonic cleaning fluid of the dentures, non-albicans Candida species such as C. glabrata were detected at a higher rate than C. albicans.

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口腔カンジダと義歯の関連に関する研究

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【目的】義歯装着と口腔カンジダの関連について検索することである。

【方法】対象は 2007 年 2 月から 2008 年 8 月までの 18か月間にかわさき歯科口腔外科医院を受診し、口腔カンジダ症の所見のない患者の内、本研究に同意した 417 名である。方法は、全対象者から舌背ぬぐい液を、義歯装着者の 76 名（143床）の義歯床下粘膜ぬぐい液を、義歯の提供を受けた 25 名の 42床から超音波洗浄後廃液を、クロムアガーカンジダ寒天培地上に播種、培養し C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, C. krusei を検出、同定した。年齢、性別、義歯装着状況などを検索した。

【結果】舌背ぬぐい液培養では女性、高齢者および義歯使用者から、義歯床下粘膜ぬぐい液培養では下顎より上顎からの検出率が高く、義歯洗浄後廃液からの検出率は舌背や義歯床下粘膜ぬぐい液からより高く、統計学的に有意であった。義歯洗浄液の培養においては C. glabrata など non-albicans Candida 種の検出率が高かった。

【結論】義歯装着と口腔カンジダの関連が示唆され、義歯床洗浄液からは C. glabrata など non-albicans Candida 種の検出率が高優勢であった。

キーワード：口腔カンジダ、義歯、口腔カンジダ症、義歯性口内炎、ノンアルビカンスカンジダ